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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 1970 for a patent by UNISEARCH LIMITED and THE UNIVERSITY OF SYDNEY filed on 08 December 2000.

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Twenty-second day of February 2002

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and
The University of Sydney

PROVISIONAL SPECIFICATION

Invention Title:

Synthesis of small particles

The invention is described in the following statement:

Technical field

The present invention relates to a method for forming fine particles of a biologically active substance, in particular, pH-sensitive proteins such as insulin or DNase, by antisolvent precipitation. The invention also relates to fine particles of a pH-sensitive, biologically active substance produced by the method and to compositions, particularly pharmaceutical compositions, containing the pH-sensitive, biologically active substance.

Background

Pulmonary absorption is an important route of entry for some pulmonary diseases, for example, bronchial asthma. One advantage of this mode of administration is that access to the circulation is rapid, because the surface area is large. As well as almost instantaneous absorption of the drug into the blood, delivery to the lung has the advantages of avoidance of hepatic first-pass loss, and in the case of pulmonary disease, local application at the desired site of action.

Therefore, delivery to the lung may provide an alternative for the treatment of conditions that have traditionally been treated by systemic administration of a drug. The administration of proteins is a case in point. Insulin is currently administered by injection because it is not stable in the gastrointestinal tract. Diabetic patients need to self-administer several injections. However, there is a lack of compliance with the use of injections because of the associated inconvenience and pain. Administration of the protein to the lung is more likely to be accepted by such patients and is therefore an attractive alternative to injections, as long as the protein can be formed as fine particles, without significant loss of biological activity. DNase is another example of a protein that is advantageously delivered to the lung. Indeed, recombinant human DNase is a commercially available protein approved for administration by nebulisation, as an inhalation aerosol, to patients with cystic fibrosis.

Essential criteria for the use of aerosol delivery for the administration of therapeutic drugs to the lung are that the drug is in particulate form with the particles having a size in the range of about 0.05-10μm, preferably 1-5 μm and that the structural stability should be maintained.

Drugs in the form of fine particles are also suitable for use in the area of controlled or sustained release delivery. One application of such technology is in the case of a drug in which there is a small difference in dosage levels between the drug being effective and being toxic. In the latter technology, it is important that the particles have a uniform particle size.

Production of uniform micron size particles of fragile molecules such as proteins is a challenge in the pharmaceutical industry.

Dense gas techniques utilizing fluids, near or above the critical point, as a solvent or antisolvent have been developed in recent years. Two dense gas methods have been considered for the production of solid particles. The first method is known as the Rapid Expansion of Supercritical Solutions (RESS), and involves expanding a supercritical solution of the material of interest through a nozzle. Whilst providing a very effective method for producing fine particles, the applicability of the RESS method is limited by the low solubility of proteins in dense carbon dioxide.

The second method, known as the gas anti-solvent process, involves rapidly precipitating solutes from organic solutions, typically using dense carbon dioxide as an anti-solvent. The anti-solvent expands the solution, thereby decreasing the solvation power of the solvent, and eventually resulting in the precipitation of the solute.

Gas anti-solvent processes have been utilized for the generation of micron-sized particles in two modes. The first mode, known simply as the gas anti-solvent process (GAS), involves the gradual addition of an anti-solvent to the organic solution containing the solute until the precipitation occurs. The second mode, known as the Aerosol Solvent Extraction System (ASES), involves continuous introduction of a solution containing the solute of interest through a nozzle into a flowing dense gas stream.

Gas anti-solvent processes have been employed for the generation of micron-sized particles of insulin, lysozyme, tyrosine, and peroxidase. The difficulty of applying these techniques to the processing of proteins is that they involve exposure of the protein to organic solvents, the latter being potential denaturants.

In order to overcome this limitation, a new supercritical fluid technique has been developed, referred to as Solution Enhanced Dispersion by Supercritical Fluid (SEDS). SEDS involves using a special coaxial nozzle. This technique facilitates the generation of bioactive particles of lysozyme

from aqueous solutions, the advantage of which is that water is the native and favourable medium for proteins.

We have found that the biological activity of some proteins may be adversely affected when CO_2 is used as the antisolvent. We believe that the dissolution of CO_2 in the aqueous solution decreases the pH of the aqueous phase to an extent that may influence the biological activity of pH-sensitive proteins such as DNase and insulin. We believe that this loss of biological activity may be mitigated by the use of an antisolvent that does not produce a pH change in the solvent used for the biologically active protein. In order to eliminate the effect of CO_2 on the pH of the aqueous solution, we used ethane modified with ethanol as the antisolvent. We found that use of this antisolvent/modifier combination did not significantly degrade the biological activity of the insulin or DNase precipitated from aqueous solution.

Disclosure of the invention

In a first aspect, the present invention provides a method of forming fine particles of a pH-sensitive, biologically active substance, said method comprising:

contacting an non-gaseous fluid of the biologically active substance with a dense fluid to expand the solution, wherein the dense fluid comprises (a) an antisolvent that does not significantly alter the pH of the non-gaseous fluid and (b) a modifying agent that modifies the polarity of the antisolvent and acts as an extractant for the non-gaseous fluid.

The method of the present invention is capable of producing fine particles of the pH-sensitive, biologically active substance, the biological activity of which is substantially retained.

The non-gaseous fluid is preferably an aqueous solution.

The antisolvent used in the method of the invention may be selected from a C_{1-4} alkane gas, a C_{2-4} alkene gas, a C_{2-4} alkyne gas or two or more thereof. Preferably the antisolvent is an alkane gas. Ethane is a particularly preferred antisolvent.

The modifying agent may be present in an amount sufficient to absorb substantially all of the non-gaseous fluid of the non-gaseous fluid-biologically active substance mixture.

The modifying agent may be any substance that modifies the polarity of the antisolvent and acts as an extractant for the non-gaseous fluid. The

modifying agent may be selected from the group consisting of C_{1-6} alkanols, C_{1-6} thiols and C_{1-6} amines. Preferably, the modifying agent is ethanol.

A particularly preferred antisolvent/modifying agent combination is ethane/ethanol.

The dense gas can be at various temperatures and pressures. Preferably the temperature of the dense gas is in the range of sub 0°C to about 100°C, most preferably about 5°C to about 40°C. Preferably the dense gas has a pressure in the range of about 1 bar to 200 bar. A pressure between about 5 to 100 bar is particularly preferred. Most preferably, the pressure of the dense fluid is such as to maintain the antisolvent and modifying agent as a single phase.

Preferably, both the antisolvent gas and the modifier are substantially inert to the pH-sensitive, biologically active substance.

The particles produced by the method of the invention may also include delivery agents such as liposomes, lipids (including phospholipids), water soluble polymers, controlled-delivery coatings, surfactants, phytosterols, and any other delivery agents known in the art.

Preferably, the fine particles produced by the method of the invention have a particle size in the range of about 0.05-10μm. More preferably, the fine particles have a size no greater than 6.5 μm. Particles having a size in the range of about 1-5 μm are particularly useful for administration to the lung. If smaller particles are desired, it is believed that the method of the present invention can produce particles down to nanometre size.

The solution of the pH-sensitive, biologically active substance may be contacted with dense gas in any suitable manner. Preferably, the solution is introduced as droplets into the dense gas. For example, the solution and dense gas may be contacted by concurrently spraying the two through a nozzle or the like. Alternatively, the solution may be sprayed through the dense gas. A further option is to pass the solution cocurrently or countercurrently with respect to a stream of the dense gas. The solution may be passed through a continuum of the dense gas in the form of a thin film or plurality of streams.

Preferably the method of the invention is carried out using the ASES process.

The term "pH-sensitive, biologically active substance", as used throughout the specification, refers to any natural or synthetic substance

which possesses a biological activity such as, for example, an enzymatic activity, channel function (e.g. ion channel), receptor or binding function, hormonal or neurotransmitter activity, or other pharmacological activity, or encodes a protein, polypeptide, peptide, peptide analog or peptidomimetic, or nucleic acid or nucleic acid in association with a protein, polypeptide or peptide, which is adversely affected by pH outside of the normal physiological pH range (e.g. 6.8 to 7.5), especially low pH (e.g. less than 5.0). The adverse affect upon the biological activity caused by the pH may be the result of, for example, degradation or conformational changes in the substance or inactivation of an active site or binding domain.

The pH-sensitive, biologically active substance is preferably selected from the group consisting of an antimicrobial agent, virus, antiviral agent, antifungal pharmaceutical, antibiotic, nucleotide, DNA, antisense DNA, RNA, antisense RNA, amino acid, peptide, protein, enzyme, hormones, immune suppressant, thrombolytic, anticoagulant, central nervous system stimulant, decongestant, diuretic vasodilator, antipsychotic, neurotransmitter, sedative, anaesthetic, surfactant, analgesic, anticancer agent, anti-inflammatory, antioxidant, antihistamine, vitamin, mineral, sterol, phytosterol, lipid and esters of fatty acids.

More preferably, the pH-sensitive, biologically active substance is selected from proteins, polypeptides, peptides, peptide analogs or peptide mimetics. Most preferably, the pH-sensitive, biologically active substance is selected from insulin and DNase.

In a second aspect, the present invention provides a pharmaceutical composition comprising particles of a pH-sensitive, biologically active substance produced by the method of the present invention.

The pharmaceutical composition is preferably in a form suitable for inhalation delivery, for example, for delivery by a metered dose inhaler or a nebuliser.

In a third aspect, the present invention provides a method of treatment of a subject, the method comprising administering to the subject, an effective amount of particles of a pH-sensitive, biologically active substance produced by the method of the present invention.

Preferably the administration of particles of a biologically active substance is by inhalation, preferably as an aerosol.

The method of the third aspect may be the treatment of insulin-dependent diabetes by administration of insulin particles produced by the method of the present invention.

The method of treatment of the third aspect may be the treatment of cystic fibrosis by administration of DNase particles produced by the method of the present invention.

The method of the present invention, in its preferred forms, may provide one or more of the following advantages:

1. The ability to produce fine powders of proteins and other pharmaceuticals with narrow particle size distributions.
2. The ability to use aqueous solutions thereby enabling concentrated solutions of material to be processed with minimal risk of deactivation of biological activity.
3. The use of the preferred antisolvent ethane overcomes the problems associated with an acidic pH environment (ethane is neutral).
4. The use of an organic compound such as ethanol as the modifier in the ethane phase may enhance the morphological characteristics of the powders produced, including insulin.
5. The ability to use ethanol as a modifier for the antisolvent as described in 4 above, yet produce fine particles of a biologically active substance in which 90 to 95% biological activity has been retained.
6. The ability to introduce an additional component in either the solvent stream or the modified antisolvent stream, which when co-precipitated with the protein or pharmaceutical will enhance dissolution rates and/or bioavailability.
7. The ability to process materials below those required for supercriticality, thereby reducing the risk of thermal degradation.
8. The ability to work at lower pressures than that claimed in the prior art, thereby reducing the potential cost of the process.

In order that the invention may be more readily understood, we provide the following non-limiting embodiments

Brief Description of Drawing

Figure 1 is a schematic diagram of an embodiment of an apparatus that may be used in the process of the present invention.

Embodiment of the invention

Example

Procedure

Referring to Fig. **1**, an ASES apparatus **10** was used in this study is shown in Figure 1. The desired mixture of ethanol with ethane was prepared in the syringe pump (ISCO Model 260) **1**. The chamber was first pressurised with carbon dioxide via a syringe pump (ISCO Model 500) **3** to attain a pressure of 20 to 180 bar to maintain the ethane ethanol mixture as a single phase. The modified ethane was then delivered into the precipitation chamber **5** at the desired processing conditions and CO_2 was purged from the system. The operating temperature was controlled to within ± 0.1 °C using a temperature controlled water bath heated by heater **7**.

A coaxial nozzle **9** was mounted on the top of the precipitation chamber **5** to facilitate simultaneous spraying of the aqueous solution of the protein **13** and the antisolvent **15** and to achieve optimum dispersion of the aqueous solution, and mixing of the two streams.

Once the desired temperature and pressure had been achieved in the chamber **5**, and the chamber was filled with ethane-ethanol mixture, the aqueous solution containing the protein was pumped from container **17** at a constant flow rate using a solvent delivery unit (Waters pump, Model 510) **19** and sprayed through a capillary nozzle (50 um ID) into the chamber. The pressure drop through the nozzle was adjusted to about 50 bar by a metering valve **21**. Modified ethane was fed continuously through to the chamber at a constant flow rate that was adjusted with a metering valve placed at the exit.

Protein particles precipitated after the expansion and extraction of the water from the droplets or protein particles precipitated when the droplet swells, and water is extracted from the droplet. After precipitation, water and ethanol residues in the protein were washed out at the operating pressure by modified ethane. The precipitation chamber was then gradually depressurised and the powder was collected for characterization.

The operating conditions, the flow-rate ratio of the aqueous feed and the antisolvent, and the modifier mole fraction were optimized to have a homogenous mixture of ethane-ethanol water in the precipitation chamber.

The mole fraction of ethanol in the antisolvent was kept at 0.3 and a volumetric flow rate ratio of feed to antisolvent of 0.4/12 was used in the

process. The flow rate of the aqueous solution was adjusted to achieve a pressure drop of 50 bar across the nozzle, thus allowing optimum atomisation of the spray as smaller droplets are generated when the solution was sprayed at a higher pressure drop. The high flow rate of the antisolvent facilitated the dispersion and mixing of the aqueous spray mist across the chamber resulting in higher rates of water extraction from the droplets.

Micronised particles of proteins with uniform particle size suitable for aerosol drug delivery systems can now be produced from aqueous solution at room temperature in one step.

In the embodiment described, no toxic chemical was used. We have found that the residual ethanol content in the final product to be less than 100ppm.

The small particulate material of the present invention is particularly useful in the preparation of devices that provide controlled or sustained release.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Further, throughout this specification, unless stated otherwise, where a document, act or item of knowledge is referred to or discussed, this reference or discussion is not an admission that the document, act or item of knowledge, or any combination thereof, at the priority date, was part of the common general knowledge.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this 8th day of December 2000

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Patent Attorneys for the Applicant:

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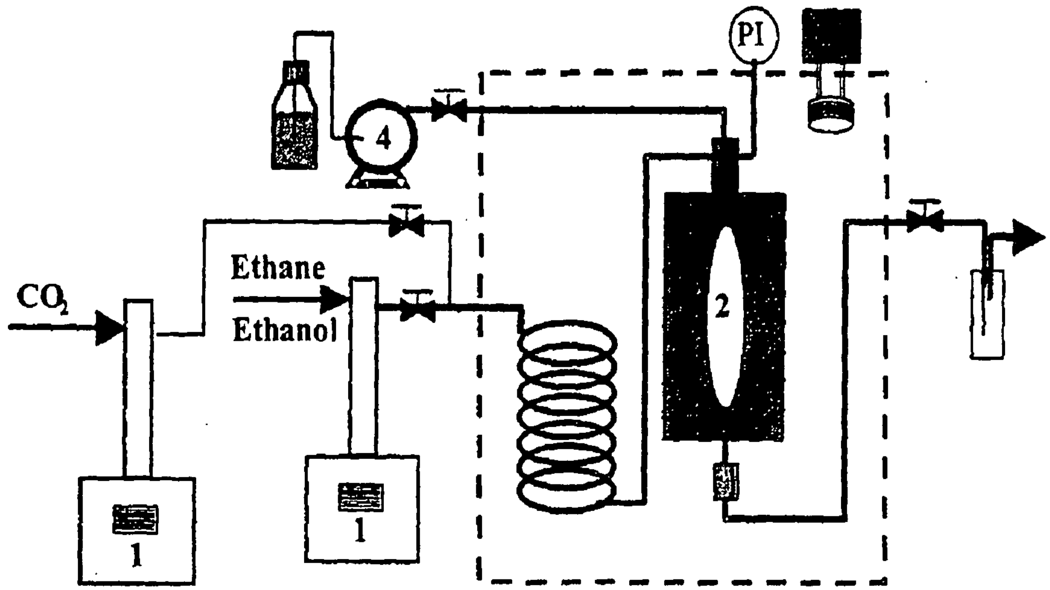

1. Syringe Pump
2. Precipitation chamber
3. Heater
4. Water pump

Fig. 1